

## SUPPLEMENTAL MATERIAL

### LEGEND TO SUPPLEMENTAL TABLES

**Table S1:** Oligonucleotide primers (Applied Biosystem) used for qRT-PCR by TaqMan

**Table S2: Evaluation of statistical significant differences through t-test analysis.** Complete list of the P-values referred to Fig.1, 2 and S1. Asterisks refer to a statistically significant difference. \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.005$ ; ns= not significant; n/a=not applicable. RBC= Red Blood Cells; Hb= Hemoglobin; Ret= reticulocytes; EPO= erythropoietin; SIC= Spleen Iron Content; LIC= Liver Iron Content.

**Table S3: Evaluation of statistical significant differences through t-test analysis.** Complete list of the P-values referred to Fig.3, 4 and S2. Asterisks refer to a statistically significant difference. \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.005$ ; ns= not significant; n/a=not applicable. RBC= Red Blood Cells; Hb= Hemoglobin; Ret= reticulocytes; EPO= erythropoietin; SIC= Spleen Iron Content; LIC= Liver Iron Content

**Table S1**

Name	Id
Hprt1	Mm01318743_m1
Hamp	Mm00519025_m1
Bmp6	Mm01332882_m1
Id1	Mm00775963_g1
Smad7	Mm03023958_m1
Atoh8	Mm00464055_m1
Tmprss6	Mm00551108_m1

**Table S2**

	Males						Females					
	<i>Tmprss6</i> <sup>-/-</sup> vs wt	<i>th3/+</i> vs wt	<i>Tmprss6</i> <sup>-/-</sup> <i>th3/+</i> vs wt	<i>th3/+</i> vs <i>Tmprss6</i> <sup>-/-</sup>	<i>Tmprss6</i> <sup>-/-</sup> <i>th3/+</i> vs <i>Tmprss6</i> <sup>-/-</sup>	<i>Tmprss6</i> <sup>-/-</sup> <i>th3/+</i> vs <i>th3/+</i>	<i>Tmprss6</i> <sup>-/-</sup> vs wt	<i>th3/+</i> vs wt	<i>Tmprss6</i> <sup>-/-</sup> <i>th3/+</i> vs wt	<i>th3/+</i> vs <i>Tmprss6</i> <sup>-/-</sup>	<i>Tmprss6</i> <sup>-/-</sup> <i>th3/+</i> vs <i>Tmprss6</i> <sup>-/-</sup>	<i>Tmprss6</i> <sup>-/-</sup> <i>th3/+</i> vs <i>th3/+</i>
RBC 1 month	ns	***	*	***	ns	*	ns	***	*	***	ns	*
RBC 2 months	ns	***	**	***	*	**	*	***	ns	***	**	***
RBC 4 months	ns	***	*	***	ns	***	ns	***	*	***	ns	***
RBC 6 months	ns	***	ns	***	ns	***	*	***	ns	***	*	***
Hb 1 month	***	***	***	***	ns	***	***	***	***	*	ns	ns
Hb 2 months	***	***	***	***	ns	***	***	***	***	*	ns	***
Hb 4 months	***	***	***	***	ns	***	***	***	***	***	ns	***
Hb 6 months	***	***	***	***	ns	*	***	***	***	*	***	*
Ret 2 months	n/a	***	***	n/a	n/a	***	n/a	***	***	n/a	n/a	***
Ret 4 months	n/a	***	***	n/a	n/a	***	n/a	***	***	n/a	n/a	*
Ret 6 months	***	***	***	***	*	***	ns	***	***	***	ns	***
CD71+/Ter119+	ns	***	*	***	*	*	ns	**	*	***	*	***
CD71-/Ter119+	ns	ns	ns	ns	ns	ns	***	***	***	ns	ns	ns
Spleen Size	ns	***	***	***	*	***	ns	***	*	***	ns	*
EPO	***	***	***	*	*	ns	*	***	**	ns	ns	ns
SIC	ns	***	ns	***	ns	***	ns	***	ns	*	ns	***
LIC	*	***	ns	***	ns	***	***	***	***	***	ns	***
Hamp	*	ns	***	*	*	***	***	ns	*	*	ns	*
Hamp/LIC	*	***	**	*	ns	*	***	***	*	***	ns	**
Bmp6	ns	***	*	***	ns	*	ns	*	*	*	*	ns
Id1	***	***	***	ns	***	*	**	ns	**	**	ns	***
Smad7	***	***	***	***	***	ns	***	ns	***	***	ns	***
Atoh8	***	**	***	***	ns	*	***	ns	***	***	ns	***

**Table S3**

	Males						Females					
	<i>Tmprss6<sup>+/+</sup></i> vs wt	<i>th3/+</i> vs wt	<i>Tmprss6<sup>+/+</sup>/th3/+</i> vs wt	<i>th3/+</i> vs <i>Tmprss6<sup>+/+</sup></i>	<i>Tmprss6<sup>+/+</sup>/th3/+</i> vs <i>Tmprss6<sup>+/+</sup></i>	<i>Tmprss6<sup>+/+</sup>/th3/+</i> vs <i>th3/+</i>	<i>Tmprss6<sup>+/+</sup></i> vs wt	<i>th3/+</i> vs wt	<i>Tmprss6<sup>+/+</sup>/th3/+</i> vs wt	<i>th3/+</i> vs <i>Tmprss6<sup>+/+</sup></i>	<i>Tmprss6<sup>+/+</sup>/th3/+</i> vs <i>Tmprss6<sup>+/+</sup></i>	<i>Tmprss6<sup>+/+</sup>/th3/+</i> vs <i>th3/+</i>
RBC 1 month	ns	***	***	***	***	***	ns	ns	***	***	***	***
RBC 2 months	ns	***	***	***	***	***	ns	ns	***	***	***	***
RBC 4 months	ns	***	***	***	***	***	ns	***	***	***	***	ns
RBC 6 months	ns	***	***	***	***	**	ns	***	***	***	*	**
Hb 1 month	*	***	***	***	***	***	ns	ns	***	***	***	***
Hb 2 months	ns	***	***	***	***	***	*	ns	***	***	***	***
Hb 4 months	ns	***	***	***	***	***	*	ns	***	***	***	ns
Hb 6 months	ns	***	***	***	***	***	*	*	***	***	***	ns
MCV 1 month	ns	ns	*	ns	*	ns	ns	ns	*	***	ns	**
MCV 2 months	*	***	***	***	***	***	ns	**	***	***	***	ns
MCV 4 months	*	***	***	***	***	***	ns	*	***	***	***	ns
MCV 6 months	ns	***	***	***	***	***	***	ns	***	***	***	ns
Spleen Size	ns	***	***	***	***	***	***	ns	***	***	***	ns
EPO	ns	***	***	***	***	***	*	ns	***	***	***	ns
SIC	ns	***	***	***	***	***	ns	ns	***	***	***	ns
LIC	*	***	***	***	***	***	*	ns	***	***	***	ns
Hamp/LIC	ns	***	***	***	***	***	***	*	***	*	***	*
Bmp6	ns	***	***	***	***	***	ns	ns	*	*	*	ns
Id1	ns	***	ns	**	ns	*	ns	ns	ns	ns	ns	ns
Tmprss6	n/a	***	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Hamp	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Smad7	ns	***	*	***	*	ns	ns	ns	ns	ns	ns	ns

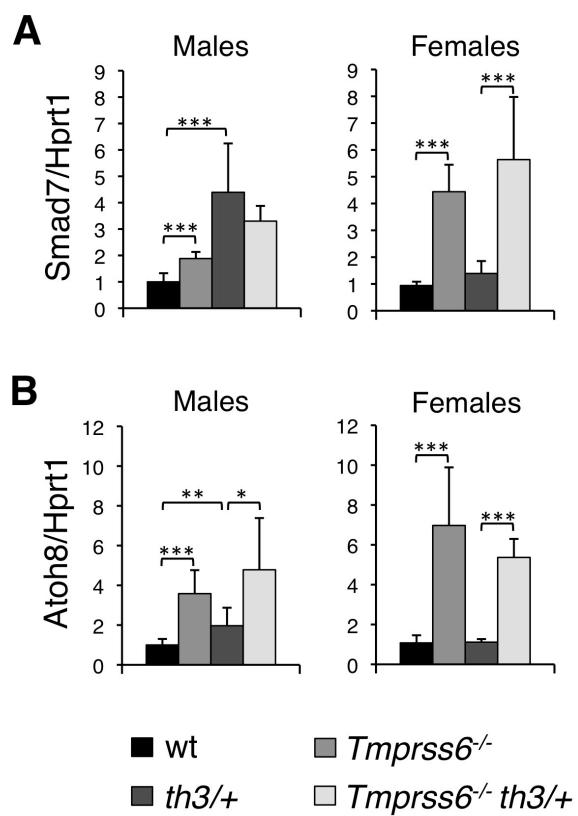
## LEGEND TO SUPPLEMENTAL FIGURES

**Figure S1: Modulation of the expression of *Smad7* and *Atoh8* in *Tmprss6<sup>-/-</sup>Hbb<sup>th3/+</sup>* mice.** Liver mRNA expression of Son of Mothers Against Decapentaplegic 7 (*Smad7*; A) and Atonal Homolog 8 (*Atoh8*; B) measured by TaqMan qRT-PCR. Hypoxanthine phosphoribosyltransferase 1 (*Hprt1*) was used as the housekeeping gene to normalize gene expression. mRNA expression ratio was normalized to a male wt mean value of 1. Mean values of 4-8 animals for gender and genotype [wt, *Tmprss6<sup>-/-</sup>*, *Hbb<sup>th3/+</sup>* (*th3/+*) and *Tmprss6<sup>-/-</sup>Hbb<sup>th3/+</sup>* (*Tmprss6<sup>-/-th3/+</sup>*)] are graphed and error bars indicate standard deviation. Asterisks refer to a statistically significant difference. \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.005$ . For complete statistical analysis see Table S2.

**Figure S2: Modulation of the expression of *Hamp* and *Smad7* in *Tmprss6<sup>+/+</sup>Hbb<sup>th3/+</sup>* mice**

Liver mRNA expression of Hepcidin (*Hamp*; A) and Son of Mothers Against Decapentaplegic 7 (*Smad7*; B) measured by TaqMan qRT-PCR. Hypoxanthine phosphoribosyltransferase 1 (*Hprt1*) was used as the housekeeping gene to normalize gene expression. mRNA expression ratio was normalized to a male wt mean value of 1. Mean values of 4-8 animals for gender and genotype [wt, *Tmprss6<sup>+/+</sup>*, *Hbb<sup>th3/+</sup>* (*th3/+*) and *Tmprss6<sup>+/+Hbb<sup>th3/+</sup></sup>* (*Tmprss6<sup>+/+th3/+</sup>*)] are graphed and error bars indicate standard deviation. Asterisks refer to a statistically significant difference. \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.005$ . For complete statistical analysis see Table S3.

**Figure S1**



**Figure S2**

